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Adenosine receptor mediates nicotine-induced antinociception in formalin test

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Abstract

In this study, the effect of adenosine receptor agents on nicotine induced antinociception, in formalin test, has been investigated. Intraperitoneal (i.p.) administration of different doses of nicotine (0.1, 1, 10 and 100 μg kg^{-1}) induced a dose-dependent antinociception in mice, in the both first and second phases of the test. Adenosine receptor antagonist, theophylline (5, 10, 20 and 80 mg kg^{-1}, i.p.) also induced antinociception in the both phases, while a dose of the drug (40 mg kg^{-1}, i.p.) did not induce any response. Theophylline reduced antinociception induced by nicotine in both phases of formalin test. The A_2 receptor agonist, 5′-*N*-ethylcarboxamide adenosine (NECA; 1 and 5 μg kg^{-1}, i.p.) also produced antinociception, which was reversed with different doses of theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.). But administration of the adenosine receptor agonist, NECA did not potentiate the response of nicotine. It is concluded that adenosine system may be involved in modulation of antinociception induced by nicotine.

Keywords: Nicotine; NECA; Theophylline; Formalin test; Mice

1. Introduction

Adenosine is a neurotransmitter which, has generally inhibitory effect on nervous system [1], so that, adenosine receptor activation inhibits neural activity in many areas along the neuroaxis [2]. Moreover, it is one of the several endogenous compounds that may have a role in nociceptive information [3], and contributes to antinociception induced by oipioids, noradrenaline, 5-hydroxytryptamine, tricyclic antidepressants and transcutaneous electrical nerve stimulation [4]. Adenosine functions through at least three subtypes of adenosine receptor: A_1, A_2 and A_3 [5,6]. These receptor sites have been pharmacologically characterized by use of adenosine agonists and antagonists [7]. Adenosine has complex effects on pain transmission at peripheral and spinal sites, due to different subtypes of adenosine receptors.

In fact, there is a controversy on the role of A_1 adenosine receptors in antinociception. Some of studies confirm the antinociceptive effect of the receptors [3,8–12], while the others show the nociceptive response induced by the A_1 adenosine receptors [13–15]. Meanwhile, it has been suggested that activation of the peripheral A_1 adenosine receptors produce pronociceptive and pain enhancing effect [4,16].

Adenosine has recently been proposed to be a significant anti-inflammatory autacoid released peripherally under conditions of inflammation [17,18]. It seems that the A_2 receptor involves in the anti-inflammatory effect [18,19]. Within the spinal cord, activation of the both A_1 and A_2 produce antinociception. Antinociceptive actions of adenosine and adenosine analogs have been shown in a wide range of tests [4]. Adenosine receptor agonists have been proved to be more

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potent in reducing hyperalgesia and allodynia than normal acute pain conditions [20,21].

The involvement of adenosine in antinociception and antiallodynia induced by opioids has also been demonstrated [22–24], and release of adenosine in the spinal cord contributes to the spinal efficacy of opioids [4].

Furthermore, nicotine, the psychoactive component of tobacco products, is widely consumed by humans [25–28]. The drug exhibits several pharmacological actions in the central and peripheral nervous systems and releases a number of neurotransmitters [29–32]. This drug is also able to activate endogenous opioid system(s) [33]. Acute nicotinic receptor stimulation activates enkephalin and beta-endorphin [34–38] release and biosynthesis in discrete brain nuclei and peripheral tissues. While, there are other reports indicating that chronic administration of nicotine reduces met-enkelphalin and beta-endorphin [37,39,40]. Moreover, the drug has been shown to induce antinociception in different tests [41–47]. The aim of this study was to investigate the effect of adenosine receptor agonist and antagonists on the antinociception induced by nicotine in mice.

2. Materials and methods

2.1. Animals

Male NMRI mice (20–30 g) were used in these experiments. They were kept 10 per cage (45 cm × 30 cm × 15 cm) at an environmental temperature of 23 ± 1 °C on a 12-h light–dark cycle. The animals had free access to food and water, except during the time of experiments. Each animal was used once only and was euthanized immediately after the experiment. The study was carried out according to institutional guideline for animal care and use.

2.2. Drugs

The following drugs (−)-nicotine base, adenosine agonist, 5′-(*N*-ethyl) carboxamido adenosine (NECA) and theophylline were purchased from Sigma–Aldrich, UK. Nicotine solutions were prepared in saline and the pH adjusted to 7.2 ± 0.1 with a small amount of NaOH and other drugs were dissolved in saline. All the drugs were injected intraperitoneally (i.p.) in a volume of 10 ml kg^{-1}.

2.3. Drug treatment

The animals were treated as follows: groups 1 and 2 received different doses of nicotine (0.1, 1, 10 and 100 μg kg^{-1}, i.p.) or theophylline (5, 10, 20, 40 and 80 mg kg^{-1}, i.p.), respectively and antinociception was assessed as described in Section 2.4. Group 3 received different doses of theophylline (5, 10 and 20 mg kg^{-1}, i.p.) in the presence or absence of lower dose of nicotine (1 μg kg^{-1}, i.p.). Group 4 received different doses of theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.) in the presence or absence of higher dose of nicotine (100 μg kg^{-1}, i.p.). Group 5 received different doses of nicotine (1, 5 and 10 μg kg^{-1}, i.p.) alone or nicotine plus NECA (1 and 5 μg kg^{-1}, i.p.). In all groups antinociception was assessed after nicotine injection.

2.4. Antinociception recording

Animals were allowed to acclimatize for 30 min before formalin injection. Twenty-five microliters of formalin (2.5%) was injected subcutaneously into the dorsal surface of the right hind paw of the mouse using a microsyringe with a 26-gauge needle. Immediately after formalin injection, animals were placed individually in a glass cylinder (20 cm wide, 25 cm long) on a flat glass floor and a mirror was arranged in a 45° angle under the cylinder to allow clear observation of the paws of the animals [48].

Pain response was recorded immediately after formalin injection for a period of 50 min. The total time (s) spent licking the injected paw during periods of 0–5 min (first phase) and 15–50 min (second phase) after formalin injection were measured as an indicator of pain.

2.5. Statistical analysis

One-way and two-way ANOVAs followed by Newman–Keuls test, were used for analysis of the data. Differences between means were considered statistically significant if $P < 0.05$. Each point is the mean ± S.E.M. of eight mice.

3. Results

3.1. Effect of nicotine or theophylline in formalin test

Fig. 1 indicates antinociception induced by nicotine in formalin test. One-way ANOVA showed that intraperitoneal injection of mice with different doses nicotine (0.1, 1, 10 and 100 μg kg^{-1}, i.p.) induced antinociception in the first [$F(4, 35) = 38.6$, $P < 0.0001$] (Fig. 1A) and second phases [$F(4, 35) = 63.6$, $P < 0.0001$] (Fig. 1B) of the test. The response of nicotine was maximum with 100 μg kg^{-1} of the drug.

Fig. 2 indicates the response of theophylline in formalin test. One-way ANOVA indicated that administration of different doses of theophylline (5, 10, 20 and 80 mg kg^{-1}, i.p.) to mice induced antinociception in the first [$F(5, 42) = 9.6$, $P < 0.0001$] (Fig. 2A) and second [$F(5, 42) = 81.6$, $P < 0.0001$] (Fig. 2B) phases of formalin test. However, increasing of the drug doses decreased the response of drug. The drug in dose of 40 mg kg^{-1}, did not induce antinociception. However, the dose of 80 mg kg^{-1}, showed antinociception.

3.2. Effect of adenosine receptor agonist or antagonist on nicotine-induced antinociception in formalin test

Fig. 3 indicates effect of theophylline in the presence or absence of lower dose of nicotine. Two-way ANOVA showed

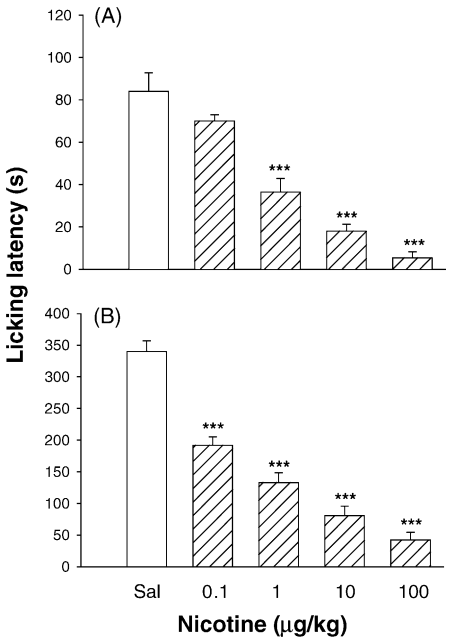

Fig. 1. Antinociceptive effect of nicotine in the formalin test. Mice were injected intraperitoneally (i.p.) either with saline (sal, 10 ml kg^{-1}) or different doses of nicotine (0.1, 1, 10 and 100 μg kg^{-1}) 15 min before formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{***}P < 0.001$ different from respective saline control group.

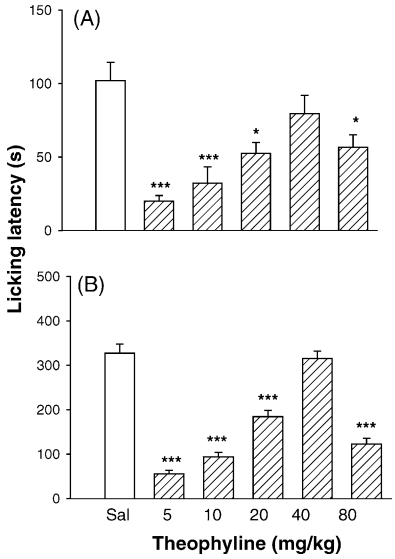

Fig. 2. Effect of theophylline in the formalin test. Animals were administered either saline (sal, 10 ml kg^{-1}) or different doses of theophylline (5, 10, 20, 40 and 80 mg kg^{-1}, i.p.) 60 min before formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{*}P < 0.05$, $^{***}P < 0.001$ different from respective saline control group.

that combination of theophylline (5, 10 and 20 mg kg^{-1}, i.p.) and lower dose of nicotine (1 μg kg^{-1}, i.p.) reduced nicotine response with interactions in the first [$F(3, 56) = 150.4$, $P < 0.0001$] (Fig. 3A) and second phase [$F(3, 56) = 107.0$, $P < 0.0001$] (Fig. 3B) of formalin test. Post hoc analysis also showed that the drugs induced antinociception in the both phases of the test.

Fig. 4 indicates effect of theophylline in the presence or absence of higher dose of nicotine. Two-way ANOVA showed that combination of theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.) and higher dose of nicotine (100 μg kg^{-1}, i.p.) reduced nicotine response with interactions in the first [$F(5, 84) = 85.7$, $P < 0.0001$] (Fig. 4A) and second phase [$F(5, 84) = 193.6$, $P < 0.0001$] of formalin test (Fig. 4B). Post-hoc analysis also showed that the drugs induced antinociception in the both phases of the test.

Fig. 5A shows the antinociception induced by different doses of nicotine in the presence or absence of NECA in the first phase of formalin test. Two-way ANOVA indicated that combination of nicotine (1, 10 and 100 μg kg^{-1}, i.p.) with NECA (1 μg kg^{-1}, i.p.) [$F(3, 56) = 40.3$, $P < 0.0001$] and also nicotine with NECA 5 μg kg^{-1} [$F(3, 56) = 39.3$, $P < 0.0001$] induced interactions. Post hoc analysis also showed that NECA did not potentiate nicotine response in first phase of the test.

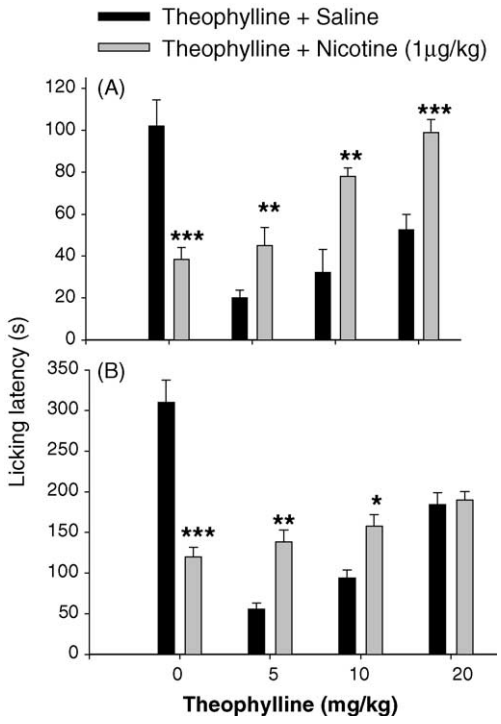

Fig. 3. Effect of theophylline in the presence or absence of lower dose of nicotine in the formalin test. Animals were administered either theophylline (5, 10 and 20 mg kg^{-1}, i.p.) 60 min before formalin injection, or theophylline plus nicotine (1 μg kg^{-1}, i.p.). Nicotine was administered 15 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ different from respective saline control group.

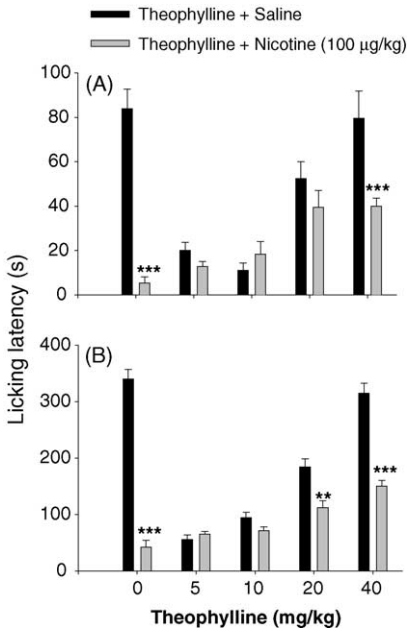

Fig. 4. Effect of theophylline in the presence or absence of higher dose of nicotine in the formalin test. Animals were administered either theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.) 60 min before formalin injection, or theophylline plus nicotine (100 μg kg^{-1}, i.p.). Nicotine was administered 15 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ different from respective saline control group.

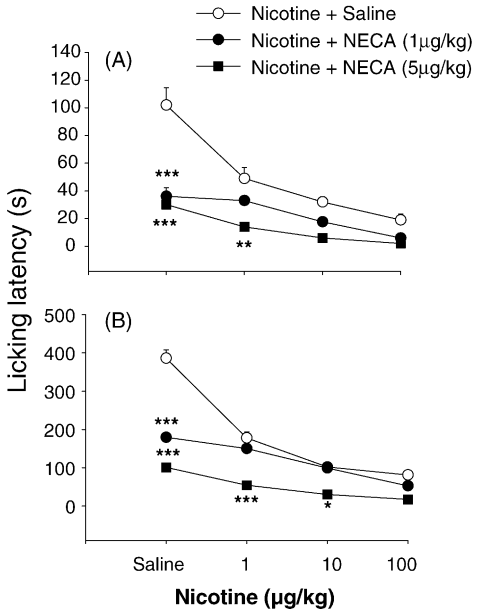

Fig. 5. Effect of nicotine in the presence or absence of 5′-*N*-ethylcarboxamide adenosine (NECA) in the formalin test. Animals were administered either nicotine (1, 10 and 100 μg kg^{-1}, i.p.) 15 min before formalin injection or nicotine plus NECA (1 and 5 μg kg^{-1}, i.p.). NECA was administered 30 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ different from respective saline control group.

Fig. 5B shows the antinociception induced by nicotine in the presence or absence of NECA in the second phase of formalin test. Two-way ANOVA indicated that combination of nicotine (1, 10 and 100 μg kg^{-1}, i.p.) and NECA 1 μg kg^{-1} [$F(3, 56) = 48.8$, $P < 0.0001$] and NECA 5 μg kg^{-1} [$F(3, 56) = 66.9$, $P < 0.0001$] induced interactions. Further analysis showed that NECA did not potentiate the response of nicotine in the second phase of the test. NECA in doses higher than 5 μg kg^{-1} (10, 50 and 100 μg kg^{-1}, i.p.) induced antinociception, which a part of the response may be due to sedation.

3.3. Effect of theophylline on adenosine-induced antinociception in formalin test

Antinociception induced by different doses of theophylline (5, 10, 20 and 50 mg kg^{-1}, i.p.) in the presence or absence of NECA 5 μg kg^{-1} is shown in Fig. 6. Two-way ANOVA showed that combination of theophylline with NECA induced interaction in the first phase (Fig. 6A) [$F(3, 56) = 48.8$, $P < 0.0001$] and second phase (Fig. 6B) [$F(3, 56) = 48.8$, $P < 0.0001$] of the formalin test. Further analysis

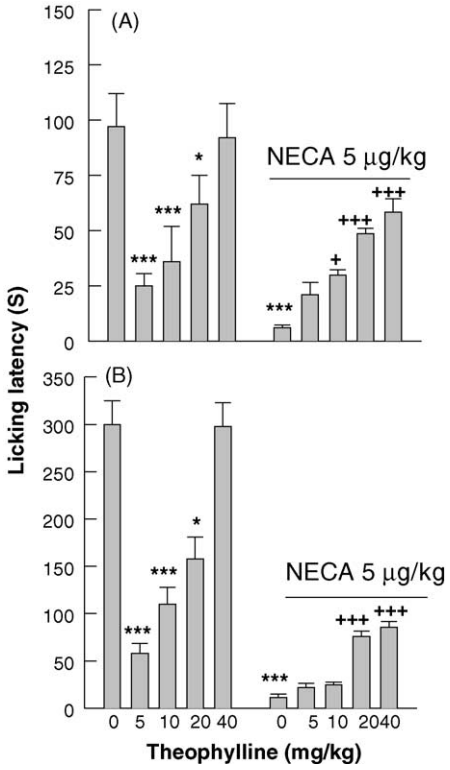

Fig. 6. Effect of theophylline in the presence or absence of 5′-N-ethylcarboxamide adenosine (NECA) in the formalin test. Animals were administered either theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.) 60 min before formalin injection or theophylline plus NECA (5 μg kg^{-1}, i.p.). NECA was administered 30 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{*}P < 0.05$, $^{***}P < 0.001$ different from respective saline control group. $^{+}P < 0.05$, $^{+++}P < 0.001$ different from respective NECA control group.

showed that theophylline reversed the antinociceptive effect of NECA.

4. Discussion

The formalin test is a model of injury-produced pain, which was introduced by Dubuisson and Dennis [49]. It measures the response to a long-lasting nociceptive stimulus; resemble clinical pain. The test produced a distinct biphasic response. The early response (first phase) was recorded during the 5 min after formalin injection, and the late response (second phase) recorded 20–50 min after formalin injection. It has been reported that the action of analgesics differs in the early phase and late phase [50–52].

The present data showed that nicotine induced a dose-dependent antinociception in the first and second phases of formalin test. This is in agreement with previous reports that nicotine induces antinociception (see Section 1). Interaction between nicotinic and opioid systems has been observed [33,36]. The antinociceptive effect of nicotine is shown to be mediated through cholinergic [42], opioid receptor [53] and GABA-A receptor mechanisms [54]. Bernardini et al. [55] showed that nicotine can weakly excite C-nociceptors, while muscarinic receptor desensitization leads to antinociception. There are also some reports indicating interaction of nicotinic and adenosine receptors [56,57]. Nicotine can cause the release of endogenous opioid peptides [33,58,59] and these peptides are able to release adenosine [60–63].

The present study also showed that theophylline, an adenosine antagonist and also a phosphodiesterase inhibitor [64], induced antinociception. However, the drug showed a dual effect. Antinociception decreased by increasing the dose of the drug to 40 mg kg^{-1} and increased again in 80 mg kg^{-1}. This is in agreement with the data showing that increase in doses of methylxanthines decreases antinociception [65,66]. As well as, it has already been shown that theophylline exerts dual effect in other experiments such as neurotransmitter release [67] or effect on morphine analgesic effect [68].

Theophylline blocks both A_1 and A_2 adenosine receptors, it can be postulated that antinociceptive effect of theophylline and decreasing its response may be due to blockade of the two different adenosine receptor subtypes. There is controversial results on role of the A_1 receptors (see Section 1). It has been reported that activation of A_1 receptors produce pronociceptive and pain enhancing effect, while A_2 receptor induces antinociceptive response. Therefore, the possibility may exist that blockade of A_1 receptor by the lower doses of theophylline induces antinociception, while the increase in doses of the drug which may block A_2 receptor, showed less response. This hypothesis is supported by our data indicating that NECA, which may have more affinity on A_2 adenosine receptor [69–71] induced antinociception, which is also in agreement by other results in this respect [72,73]. Therefore the antinociceptive effect of NECA was reversed by higher doses of theophylline (20 and 40 mg kg^{-1}) in the present study.

In the present study, the effects of nicotine in the presence or absence of theophylline or adenosine receptor agonist, NECA have been investigated in the formalin test. However, adenosine mechanism may be involved in the nicotine response, our present data showed that NECA did not potentiate the response of nicotine and the additive effect may be involved in the response of combination of the two drugs.

On the other hand, combination of nicotine with theophylline elicits lower antinociceptive effect, which can support adenosine receptor mechanism in the antinociceptive response of nicotine. This data even further supports the hypothesis that blockade of the A_2 receptors by theophylline decreases antinociception. In addition to inhibition of phosphodiesterase and 5′-nucleotide, methylxanthines are reported to have a variety of actions unrelated to their antagonism of adenosine receptors, including alterations in intracellular Ca^{2+} concentrations and modulation of GABA or noradrenergic transmission [74,75]. Whether, these mechanisms are involved in the present theophylline effects, it should be examined. Overall, it is concluded that adenosine receptor mechanism may be involved in modulation of nicotine-induced antinociception.

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